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Morphological Variation and Classification of *Nuphar* with Special Reference to Populations in Central to Western Japan

TAKASHI SHIGA[1] and YASURO KADONO[2]

[1]*Division of Life Science, Graduate School of Science and Technology, Kobe University, Nada, Kobe 657-8501, Japan;* [2]*Department of Biology, Faculty of Science, Kobe University, Nada, Kobe 657-8501, Japan*

Morphological variation in 52 populations of *Nuphar japonica* DC., *N. subintegerrima* (Casp.) Makino, *N. oguraensis* Miki and unidentified intermediate plants were investigated during a revision of *Nuphar* (Nymphaeaceae) in Japan. The phenogram of cluster analysis based on 15 morphological characters revealed five cluster groups (A～E). Clusters A, C and E had some remarkable characters and corresponded well to the description of *N. japonica*, *N. oguraensis* and *N. subintegerrima sensu stricto*, respectively. In contrast, Clusters B and D showed intermediate values in most of their characters. It was concluded that the intermediate plants, which usually had been identified as *N. subintegerrima*, were not conspecific with *N. subintegerrima s. s.* and that there were two different intermediate groups in central to western Japan. Morphological relationships suggested that the two intermediate groups were of hybrid origin between *N. japonica* and *N. oguraensis* and between *N. japonica* and *N. subintegerrima*, respectively. Results from the present study proved that populations of *N. subintegerrima s. s.* are limited in number and distribution, and that the species is one of the most endangered plants in Japan.

Key words: cluster analysis, hybridization, intermediate forms, morphological variations, *Nuphar japonica*, *Nuphar oguraensis*, *Nuphar subintegerrima*, phenetic relationships

Species of *Nuphar* Sm. (Nymphaeaceae), yellow water-lilies, are perennial freshwater aquatic macrophytes of north temperate distribution. The genus is one of the most problematic aquatic macrophytes to identify and 7 to 20 species have been recognized worldwide, according to Cook (1990), Padgett (1999) and Padgett *et al.* (1999). In Japan, four species with one or two varieties or forms have been recognized in various Japanese floras (Kitamura & Murata 1961; Ohwi 1965; Tamura 1982). In 1991, Shimoda described two new varieties in two species. Kadono (1994) enumerated four species, *N. japonica* DC., *N. oguraensis* Miki, *N. subintegerrima* (Casp.) Makino and *N. pumila* (Timm) DC. with three varieties and one form. The former three species are endemic to eastern Asia and *N. pumila* is distributed widely in the Old World (Beal 1956; Ohwi 1965; Tamura 1982; Kadono 1994).

Kadono (1994, 1995) reported intermediate forms that were difficult to identify to known species in Japan. He also addressed the problem regarding the delimitation of *Nuphar subintegerrima* (Casp.) Makino. *Nuphar subintegerrima* was first described as *N. japonica* DC. var. *subintegerrima* Casp. (Caspary 1866). Makino (1910) later recognized it as a distinct species, *N. subintegerrima* (Casp.) Makino. Makino described *N. subintegerrima* as being a dwarf plant with emergent or floating leaves 5-11 cm long and 4-8.5 cm wide. Plants with float-

ing leaves more 20 cm long, however, which are widely distributed in central to western Japan, have also been treated as *N. subintegerrima*. Kadono (1994) doubted that these plants were conspecific with *N. subintegerrima* in the strict sense as described by Makino (1910). Furthermore, some plants are intermediate in size and morphology between *N. subintegerrima s. l.* and *N. japonica*. Because herbarium specimens of *N. subintegerrima* are difficult to distinguish from *N. japonica*, Padgett (1999) and Padgett *et al.* (2002) treated *N. subintegerrima* as a synonym of *N. japonica*.

During a revision of the genus in Japan, we studied the morphology, ecology and genetics of the Japanese populations of *Nuphar*. In this first paper we describe the morphological variation, with special reference to the populations in central to western Japan where delimitation of the species of *Nuphar* is most problematic.

Materials and Methods

Sampling

Plant materials of *Nuphar* were collected from 48 localities from central to western Japan (Table 1). Some northern populations were included to represent typical *N. japonica* for comparison. Sampling was conducted from late July to early October in 2001 and 2002. In each of the three localities where we recognized apparently different forms of

TABLE 1. Localities of 52 population of Japanese *Nuphar* in this study.

Population Code	Sampling locality	Population Code	Sampling locality
AO-1	Aomori Pref.: Dekijima, Kizukuri Town	HY-5	Hyogo Pref.: Abiki-cho, Kasai C.
AK-1	Akita Pref.: Kamikitatesaruta, Akita City	HY-7	Hyogo Pref.: Tamaoka-cho, Kasai C.
YA-1	Yamagata Pref.: Tazawa, Murayama C.	HY-8	Hyogo Pref.: Namita, Sanda C.
FS-1	Fukushima Pref.: Shidahama, Inawashiro T.	OK-1	Okayama Pref.: Mitsuishigokoku, Bizen C.
FS-2	Fukushima Pref.: Nozawa, Iitate Villege	OK-2	Okayama Pref.: Kojimashirao, Kurashiki C.
FS-3	Fukushima Pref.: Hiranumanouchi, Iwaki C.	OK-3	Okayama Pref.: Kojimashirao, Kurashiki C.
IB-1	Ibaragi Pref.: Takasaki, Tamari V.	OK-4	Okayama Pref.: Kosaka, Saeki T.
IB-2	Ibaragi Pref.: Shimone-cho, Ushiku C.	OK-6	Okayama Pref.: Sugisawa, Saeki T.
NI-1	Niigata Pref.: Niihana, Toyosaka C.	HI-2a	Hiroshima Pref.: Kamitsuda, Seranishi T. (A)
NI-3	Niigata Pref.: Iwanokoshinden, Ogata T.	HI-2b	Hiroshima Pref.: Kamitsuda, Seranishi T. (B)
NI-4	Niigata Pref.: Nagamine, Yoshikawa T.	HI-7	Hiroshima Pref.: Oguni, Seranishi T.
NI-5	Niigata Pref.: Ikenodaira, Ojiya C.	KA-1	Kagawa Pref.: Sakamoto, Hiketa T.
FU-1	Fukui Pref.: Ikenokouchi, Turuga C.	KA-4	Kagawa Pref.: Nishiwake, Ayakami T.
FU-2	Fukui Pref.: Hiraizumi-cho, Katuyama C.	KA-5	Kagawa Pref.: Tomikuma, Ayauta T.
FU-3	Fukui Pref.: Hiiragi, Kanatsu T.	TO-1	Tokushima Pref.: Taura-cho, Komatsushima C.
GI-1	Gifu Pref.: Tachibokubora, Gifu C.	TO-2	Tokushima Pref.: Kandase-cho, Komatsushima C.
GI-2	Gifu Pref.: Higashitabirako, Kani C.	TO-3a	Tokushima Pref.: Shibahu-cho, Komatsushima C. (A)
GI-3	Gifu Pref.: Itoshiro, Shiratori T.	TO-3b	Tokushima Pref.: Shibahu-cho, Komatsushima C. (B)
AI-2	Aichi Pref.: Ikenodai, Inuyama C.	FO-1	Fukuoka Pref.: Ikenoyama, Hoshino V.
SI-1	Shiga Pref.: Warasono, Shinasahi T.	OH-1	Ohita Pref.: Minamiusa, Usa C.
SI-2	Shiga Pref.: Hamabun, Imazu T.	MY-1a	Miyazaki Pref.: Nagai, Kitagawa T. (A)
MI-1	Mie Pref.: Oshihuchi, Nansei T.	MY-1b	Miyazaki Pref.: Nagai, Kitagawa T. (B)
MI-2	Mie Pref.: Katsuta, Tamaki T.	MY-1c	Miyazaki Pref.: Nagai, Kitagawa T. (C)
NA-1	Nara Pref.: Hokkeji-cho, Nara C.	MY-2	Miyazaki Pref.: Nagai, Kitagawa T.
HY-1	Hyogo Pref.: Ota-cho, Ono C.	MY-6	Miyazaki Pref.: Toyomitsu-cho, Miyakonojo C.
HY-3	Hyogo Pref.: Tadokoro, Goshiki T.	MY-8	Miyazaki Pref.: Takagi-cho, Miyakonojo C.

TABLE 2. Twenty seven morphological characters investigated. Characters marked with asterisk were used for cluster analysis.

Emergent and floating leaf	
L1	Leaf blade length (cm)*
L2	Leaf blade width (cm)
L3	Leaf blade shape(L1/L2)*
L4	Sinus depth (cm)
L5	Sinus/length of blade ratio (L4/L1)
L6	Length to the maximum blade width position from the blade (cm)
L7	Maximum blade width position/total length of blade ratio (L6/L1)*
L8	Petiole diameter at 5 cm from the base of blade (mm)
L9	Presence or absence of central lacuna in petiole*: 0 = present, 1 = absent
Flower	
Fl1	Maximum length of stigmatic disk (mm)*
Fl2	Minimum length of stigmatic disk (mm)
Fl3	Min./max. ratio of disk length (Fl2/Fl1)*
Fl4	Dentation on the margin of stigmatic disk : 0 = entire, 1 = dentate
Fl5	Stigma length (mm)
Fl6	Stigma width (mm)*
Fl7	Length/width ratio of stigma (Fl5/Fl6)*
Fl8	Number of stigma
Fl9	Apical shape of stigma*: 1 = roundish, 2 = obtuse, 3 = acute
Fl10	Anther length (mm)*
Fl11	Filament length (mm)
Fl12	Anther length/filament length ratio (Fl10/Fl11)*
Fruit	
Fr1	Fruit length (mm)*
Fr2	Fruit width (mm)
Fr3	Length/width ratio of fruit (Fr1/Fr2)*
Fr4	Seed length (mm)*
Fr5	Seed width (mm)*
Fr6	Length/width ratio of seed (Fr4/Fr5)

plants growing in neighboring stands we collected the different forms separately and designated them A, B and C (Table 1). As a result, we sampled 52 populations in total. They included populations of *N. japonica*, *N. subintegerrima sensu lato*, *N. oguraensis* and the unidentified intermediate plants.

Morphological analysis

For measurement of morphological characters, more than ten emergent or floating leaves, flowers and fruits were collected from each population. Twenty-seven morphological characters, comprising 9 vegetative, 12 floral and 6 fruit characters, that have been used as important characters for the identification of the taxa of *Nuphar* (Ohtaki & Ishido 1980; Tamura 1982; Kadono 1994; Padgett *et al.* 1999) were investigated (see Table 2). Qualitative features (L9, Fl4 and Fl9) were scored as shown in Table 2 and treated as quantitative characters.

All measurements were made using materials fixed in FAA (ethanol - formalin - acetic acid) in the field.

Data analyses

Most quantitative data were continuous for all characters, making them difficult to use for recognizing taxonomic units based on specific key characters. Cluster analysis was therefore carried out for the 52 populations based on the averages of each character. The Ward method was chosen for this analysis, using standardized variables and squared Euclidean distance.

Before applying cluster analysis, a principal components analysis (PCA) was performed to select the morphological characters to use in the cluster analysis. When a character showed a high correlation ($r > 0.7$) with other characters, the character that showed the highest proportion on the first three principal components was selected. As a result, the following 15 characters were used for cluster analysis:

leaf blade length (L1),
leaf blade shape (L3),
distance to maximum width of blade/total length of blade ratio (L7)
presence or absence of central lacuna in petiole (L9)
maximum length of stigmatic disk (Fl1)
min./max. ratio of stigmatic disk length (Fl3)
stigma width (Fl6)
stigma length/width ratio (Fl7)
shape of stigma (Fl9)
anther length (Fl10)
anther length/filament length ratio (Fl12)
fruit length (Fr1)
length/width ratio of fruit (Fr3)
seed length (Fr4)
seed width (Fr5).

Differences among populations and among cluster groups for each morphological trait were tested using nested analysis of variance (nested ANOVA). When the differences were significant ($P < 0.05$), a multiple comparison was performed using Scheffe's F-test. To analyze morphological relationships among each cluster groups, PCA was performed, based on the same dataset used for the cluster analysis. All statistical tests were performed using JMP ver. 4J (SAS Institute Inc., USA).

Results

The Ward method phenogram showed three major clusters (Fig. 1). An additional subcluster was recognized within each of the second and the third clusters. These five cluster groups were recognized as operational taxonomic units (OTUs) and named Clusters A, B, C, D and E.

Significant differences among the cluster groups and populations were found in all of the measured variables by the nested ANOVA ($P < 0.05$; Table 3). Cluster A was significantly different from the other cluster groups in 17 characters. It had the highest values in 14 characters (L1-L4, L6-L8, Fl1, Fl2, Fl5, Fl8, Fl10, Fr1, Fr2) and the lowest values in two characters (L5, Fl3). Cluster C was significantly different in 13 characters and had the lowest values in six characters (L7-L9, Fl6, Fl10, Fl12). Except in only one sample, this group was especially distinct in the central lacuna in the petiole ($L9 = 0.02 \pm 0.14$). Cluster E was significantly different from other cluster groups in 19 characters. Cluster E had the highest values in four characters (Fl6, Fl12, Fr4, Fr5) and the lowest in 14 characters (L1-L4, L6, Fl1, Fl2, Fl4, Fl5, Fl7-Fl9, Fl11, Fr1). Its roundish leaves ($L3 = 1.12 \pm 0.06$ and $L7 = 0.55 \pm 0.03$) was useful for distinguishing Cluster E from the others groups, although some plants of Cluster C had roundish leaves.

Cluster B was significantly different in 12 characters and Cluster D was significantly different in 11 characters. But Clusters B and D did not have diagnostic characters, except for Fr3 and Fr6 in Cluster D. The values for most characters in both groups were intermediate between Clusters A, C and E. The values of Cluster B were intermediate between Clusters A and C in 17 characters (L1-L8, Fl1, Fl2, Fl5, Fl6, Fl8, Fl10, Fl12, Fr5, Fr6),

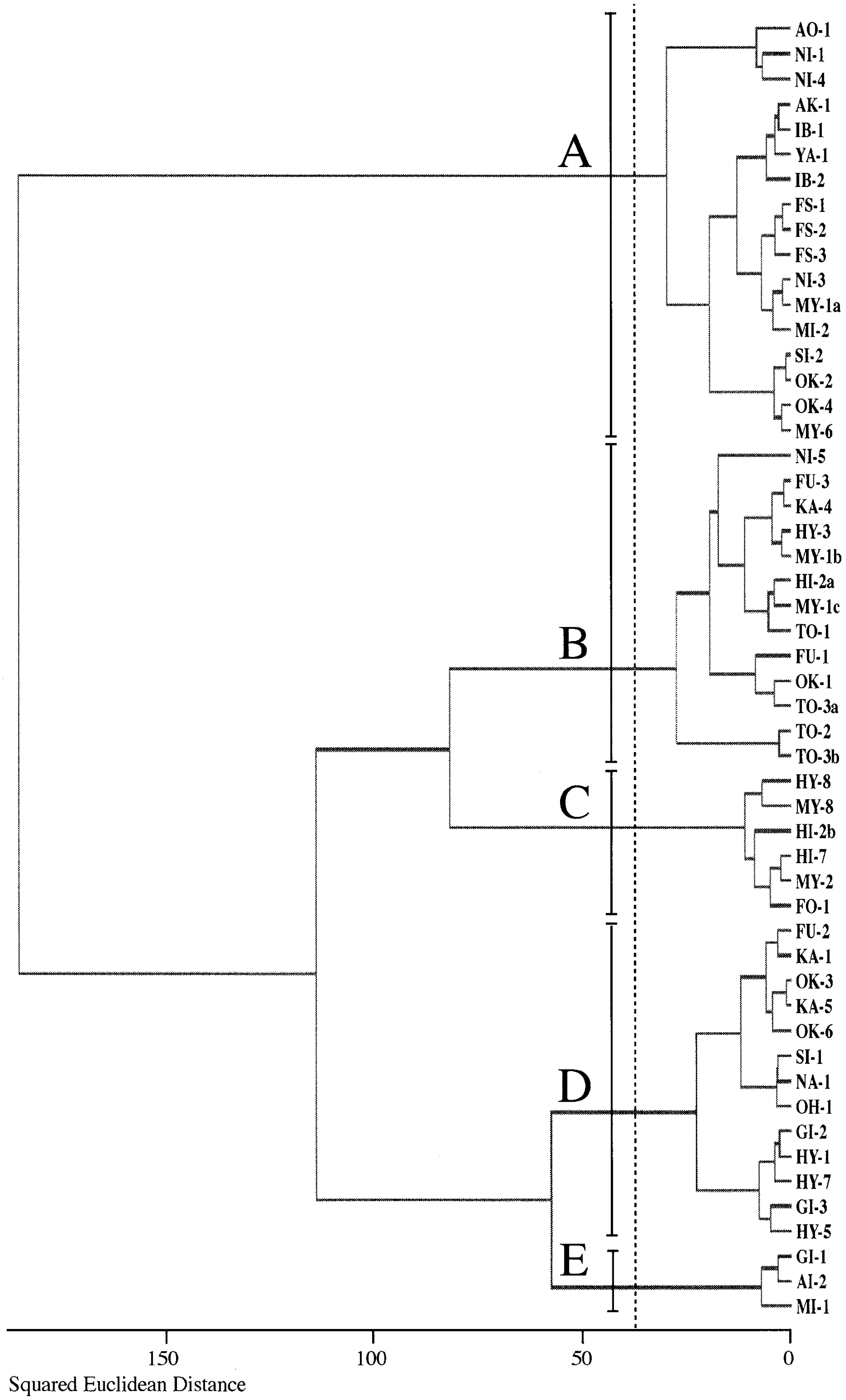

FIG. 1. Phenogram of cluster analysis of 52 *Nuphar* populations based on 15 morphological characters (Ward method).

TABLE 3. Comparison of morphological characters for five cluster groups (mean ± S.D.). Different letters indicate significant differences by Tukey HSD multiple comparison test ($P < 0.05$).

A. Emergent and floating leaf characters

	Cluster name																			
	A				B				C				D				E			
Character	mean	±	S.D.		mean	±	S.D.		mean	±	S.D.		mean	±	S.D.		mean	±	S.D.	
L1 (cm)	30.7	±	4.72	a	20.8	±	4.26	b	14.3	±	2.09	c	18.8	±	3.28	d	11.6	±	2.90	e
L2 (cm)	18.4	±	2.58	a	13.5	±	2.33	b	11.2	±	1.47	c	12.7	±	2.64	b	10.4	±	2.53	d
L3 (L1/L2)	1.68	±	0.18	a	1.54	±	0.15	b	1.28	±	0.10	c	1.51	±	0.21	d	1.12	±	0.06	e
L4 (cm)	8.1	±	1.30	a	6.6	±	0.97	b	6.1	±	0.78	c	6.1	±	1.00	c	4.8	±	0.80	d
L5 (L4/L1)	0.27	±	0.04	a	0.32	±	0.05	b	0.43	±	0.02	c	0.32	±	0.03	b	0.42	±	0.04	c
L6 (cm)	20.4	±	3.75	a	12.7	±	3.28	b	7.5	±	1.31	c	11.6	±	2.21	d	6.4	±	1.77	e
L7 (L6/L1)	0.66	±	0.06	a	0.61	±	0.05	b	0.53	±	0.03	c	0.61	±	0.05	b	0.55	±	0.03	d
L8 (mm)	9.1	±	1.36	a	6.1	±	1.59	b	3.3	±	0.89	c	5.9	±	1.22	b	4.0	±	1.12	d
L9	1.00	±	0.00		1.00	±	0.00		0.02	±	0.14		1.00	±	0.00		1.00	±	0.00	
Sample no.	20.5				222				54				313				62			

B. Flower characters

	Cluster name																			
	A				B				C				D				E			
Character	mean	±	S.D.		mean	±	S.D.		mean	±	S.D.		mean	±	S.D.		mean	±	S.D.	
F11 (mm)	10.3	±	1.71	a	8.2	±	1.35	b	7.0	±	1.29	c	7.5	±	1.35	c	6.3	±	0.79	d
F12 (mm)	9.2	±	1.51	a	7.7	±	1.24	b	6.6	±	1.08	c	7.0	±	1.29	c	6.0	±	0.79	d
F13 (F12/F11)	0.90	±	0.09	a	0.94	±	0.05	b	0.94	±	0.05	b	0.93	±	0.05	b	0.95	±	0.04	b
F14	0.94	±	0.18	a	0.94	±	0.17	a	0.97	±	0.12	a	0.85	±	0.31	b	0.73	±	0.33	c
F15 (mm)	4.0	±	0.66	a	3.3	±	0.55	b	2.8	±	0.52	c	3.0	±	0.62	c	2.5	±	0.33	d
F16 (mm)	1.0	±	0.14	a	0.8	±	0.13	b	0.7	±	0.17	c	1.0	±	0.19	a	1.2	±	0.15	d
F17 (F15/F16)	4.36	±	1.20	ab	4.14	±	0.92	a	4.10	±	1.04	b	3.08	±	0.58	c	2.11	±	0.37	d
F18 (no.)	13.74	±	2.55	a	12.14	±	1.92	b	10.48	±	1.57	c	11.42	±	2.37	d	8.37	±	1.27	e
F19 2.96	2.96	±	0.14	a	2.98	±	0.07	a	2.75	±	0.44	b	2.75	±	0.36	b	2.21	±	0.37	c
Sample no.	142				109				55				118				30			
F110 (mm)	5.4	±	0.73	a	4.4	±	0.75	b	3.2	±	0.39	c	4.7	±	0.72	d	4.2	±	0.75	b
F111 (mm)	5.8	±	0.91	a	6.2	±	1.28	b	6.5	±	1.50	b	5.2	±	1.01	c	4.4	±	1.18	d
F112 (F110/F111)	0.94	±	0.14	a	0.73	±	0.16	b	0.51	±	0.08	c	0.92	±	0.17	a	1.02	±	0.24	d
Sample no.	162				130				60				129				30			

C. Fruit characters

	Cluster name																			
	A				B				C				D				E			
Character	mean	±	S.D.		mean	±	S.D.		mean	±	S.D.		mean	±	S.D.		mean	±	S.D.	
Fr1 (mm)	41.9	±	6.23	a	32.4	±	5.53	b	32.3	±	4.99	b	31.2	±	4.92	b	28.5	±	5.47	c
Fr2 (mm)	30.5	±	4.21	a	22.0	±	5.04	b	20.7	±	3.61	b	24.1	±	3.86	c	20.2	±	3.04	b
Fr3 (Fr1/Fr2)	1.39	±	0.20	a	1.53	±	0.30	b	1.58	±	0.19	b	1.31	±	0.21	c	1.42	±	0.21	a
Fr4 (mm)	4.8	±	0.30	a	4.8	±	0.44	b	4.5	±	0.31	b	4.6	±	0.41	b	5.7	±	0.35	c
Fr5 (mm)	3.6	±	0.31	a	3.3	±	0.45	b	2.9	±	0.28	c	3.7	±	0.38	a	4.3	±	0.35	d
Fr6 (Fr4/Fr5)	1.36	±	0.10	a	1.47	±	0.14	b	1.55	±	0.17	c	1.26	±	0.09	c	1.33	±	0.11	a
Sample no.	139				96				51				118				25			

between Clusters A and E in 13 characters (L1-L8, Fl1, Fl2, Fl5, Fl8, Fr1) and between Clusters C and E in four characters (Fl6, Fl12, Fr5, Fr6). The values of cluster D were also intermediate between Clusters A and C in 10 characters (L1-L3, L5-L8, Fl8, Fl10, Fr2), between Clusters A and E in 19 characters (L1-L8, Fl1, Fl2, Fl4, Fl5, Fl7-Fl11, Fr1, Fr2) and between Clusters C and E in six characters (Fl4, Fl6, Fl7, Fl11, Fl12, Fr5).

The PCA based on the same data set with cluster analysis provided well-expressed morphological relationships among five cluster groups (Fig. 2). The two intermediate cluster groups were separated by principal component 2. Cluster B was intermediate between Clusters A and C and Cluster D was intermediate between Clusters A and E. The first three principal components were responsible for 68.5% of the variance. Principal component 1 accounted for 37.4% of total variance, which was based on leaf characters (L1, L3, L7, L9), characters of the stamens (Fl10, Fl12) and fruit length (Fr1).

TABLE 4. Loadings of 15 morphological characters for the first three factors from the analysis of 52 populations of *Nuphar*.

Characters	PCA 1	PCA 2	PCA 3
L1	0.8848	0.2381	-0.0423
L3	0.7840	0.3377	0.1413
L7	0.8311	-0.0602	0.0638
L9	0.6335	-0.3639	0.2433
Fl1	0.8207	0.3461	-0.0913
Fl3	-0.4259	-0.1377	0.5294
Fl6	0.2950	-0.8234	-0.1975
Fl7	0.2544	0.8055	0.0539
Fl9	0.4146	0.6626	0.3978
Fl10	0.8778	-0.1412	0.0263
Fl12	0.6320	-0.5681	0.2187
Fr1	0.6811	0.2461	-0.5330
Fr3	-0.3631	0.4036	-0.2587
Fr4	0.1544	-0.3653	-0.5573
Fr5	-0.3989	0.6414	-0.2343
Eigenvalue	5.6169	3.3224	1.3388
Variance %	37.44%	22.14%	8.93%
Cumulative % of variance	37.44%	59.59%	68.52%

Principal component 2, accounting for 22.1% of total variance, was contributed by characters of the stigma (Fl6, Fl7, Fl9) and the width of the seed (Fr5) (Table 4).

The geographic distribution patterns of each cluster group are shown in Fig. 3. Populations of Cluster A are widely distributed from northern Japan to Kyushu. Populations of Clusters B and D are distributed from central to western Japan. Populations of Cluster C are in western Japan. The distribution range of Cluster E is limited to the Tokai Region of central Japan.

Discussion

The phenogram based on 15 morphological characters revealed five cluster groups. The cluster groups are well distinguishable each other by many morphological characters. Clusters A, C and E in particular exhibit some remarkable characters. In contrast, Clusters B and D showed intermediate values in most of their characters.

Plants of Clusters A, C and E correspond well to the classical descriptions of *Nuphar japonica*, *N. oguraensis* and *N. subintegerrima*, respectively (De Candolle 1821; Makino 1910; Miki 1934). Cluster A includes the biggest plants in many quantitative characters and, therefore, it is obviously identifiable as *N. japonica*, one of the largest species of *Nuphar* in the Old World (Padgett *et al.* 1999; Padgett 2003). Cluster C is characterized by the thin petiole (L8 = 3.33 ± 0.89), the presence of a central lacuna in the petiole (L9 = 0.02 ± 0.14) and the lowest anther to filament length ratio (Fl12 = 0.51 ± 0.08) and is identifiable as *N. oguraensis* (Miki 1934). Cluster E includes the smallest plants with roundish leaves. Small plants with roundish leaves correspond to the description of *N. subintegerrima* by Makino (1910). Hereafter, this taxon is expressed as *N. subintegerrima sensu stricto*.

Recent taxonomic studies of worldwide *Nuphar* by Padgett (1999, 2003) and Padgett *et al.*

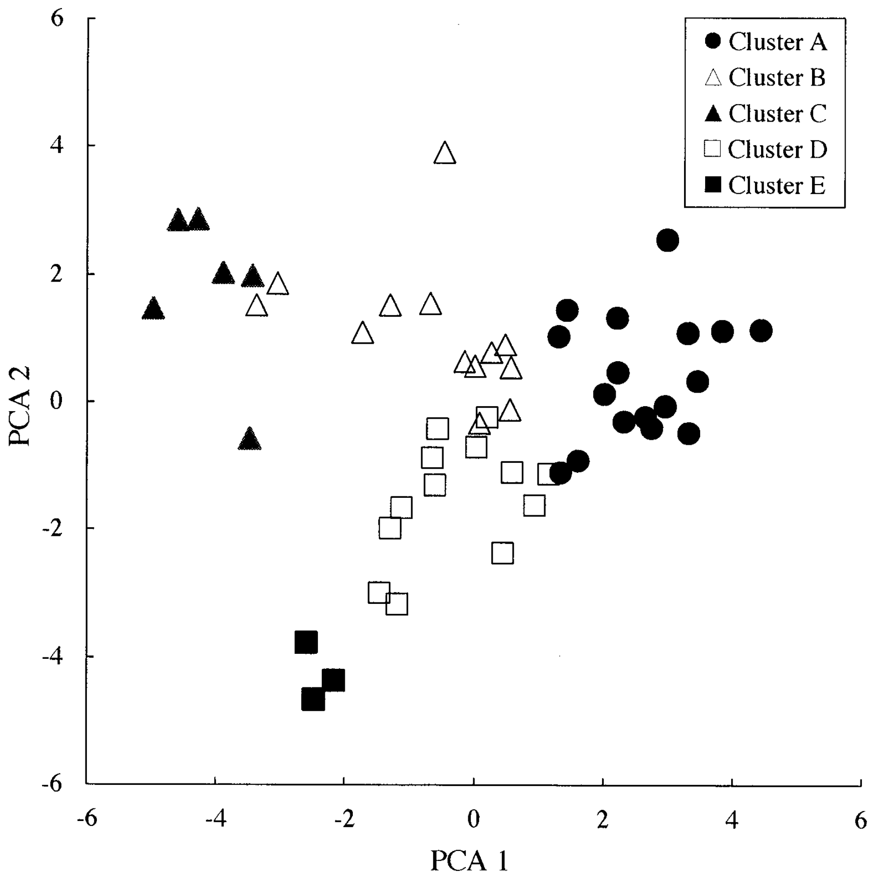

FIG. 2. 2-D scatter plot by principal components 1 and 2 of a principal component analysis using same data set as in cluster analysis.

(2002) recognized only two species in Japan, *Nuphar japonica* and *N. pumila*. They treated *N. oguraensis* as a subspecies of *N. pumila* and *N. subintegerrima* as a synonym of *N. japonica*. Our study shows that three species are easily distinguishable. *Nuphar subintegerrima s. s.* is distinct in the small, roundish leaves. Intermediate plants, which have so far been identified as *N. subintegerrima*, are not conspecific with *N. subintegerrima s. s.* The intermediate plants may have caused confusion in the treatments by Padgett (1999) and Padgett *et al.* (2002). Our preliminary allozyme analysis also showed the presence of unique alleles in *N. oguraensis* and *N. subintegerrima s. s.* (Shiga & Kadono, unpublished data).

As can be seen from the phenetic relationships among the cluster groups (Table 3, Fig. 2), the occurrences of two groups of intermediate plants, Clusters B and D, is confirmed. Although the morphological variables of the two cluster groups widely overlap, some floral features differ from each other. Plants of Cluster B are intermediate between *Nuphar japonica* and *N. oguraensis* in some floral characters (Fl1, Fl2, Fl5, Fl6, Fl8, Fl10, Fl12). Cluster D is intermediate between *N. japonica* and *N. subintegerrima s. s.* in characters Fl1, Fl2, Fl4, Fl5, and Fl7-Fl11. The PCA indicated that Cluster B was intermediate between Clusters A and C and Cluster D was intermediate between Clusters A and E. Principal component 2, summarizing the variation in some floral characters, provided evidence for differentiation between Clusters B and D. These findings support the recognition of two different intermediate groups by their morphology and suggest a hybrid origin for them from different parental combinations; Cluster B between *N. japon-*

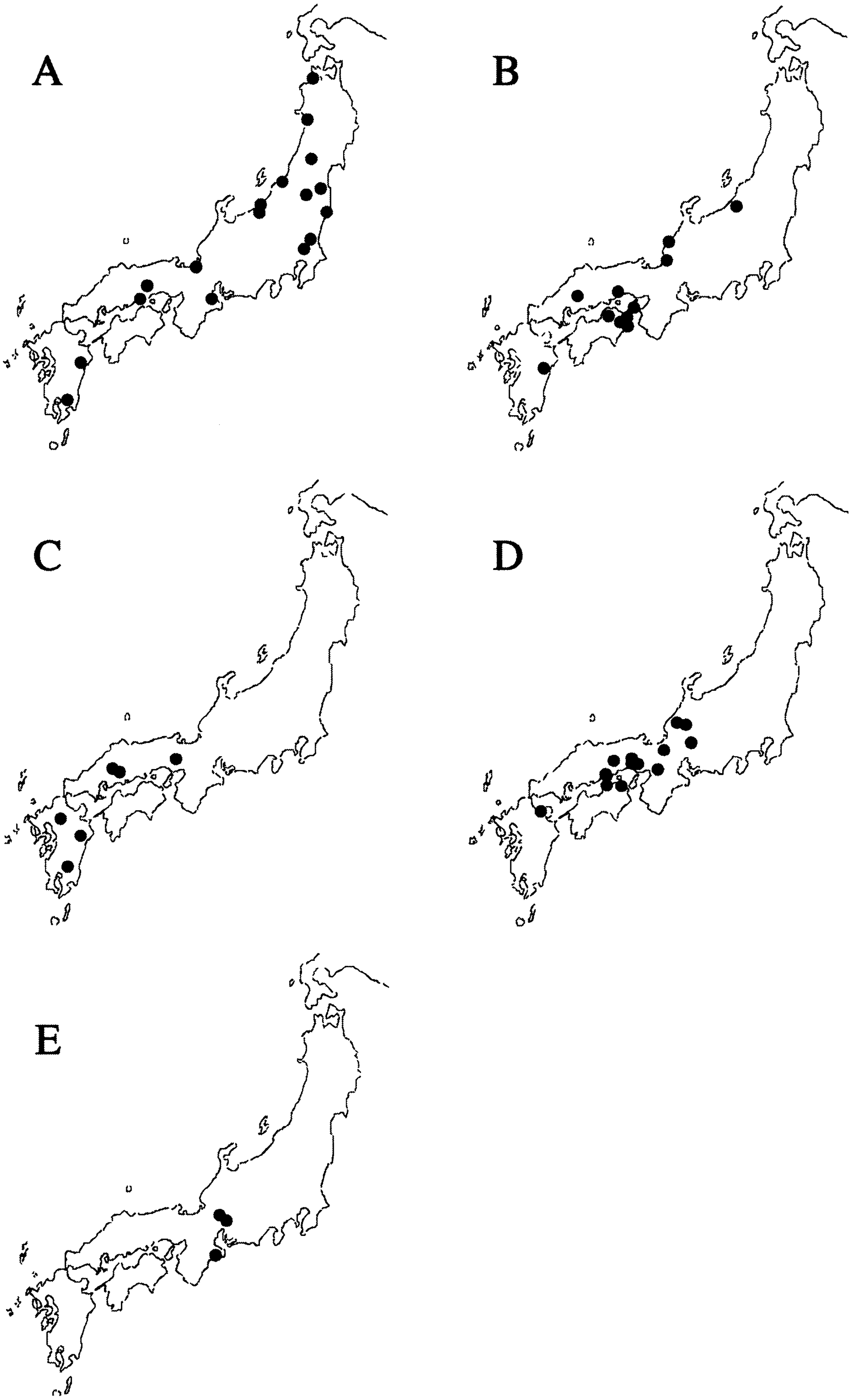

FIG. 3. Distribution of populations of five cluster groups. A. Cluster A; B. Cluster B; C. Cluster C; D. Cluster D; E. Cluster E.

ica and *N. oguraensis* and Cluster D between *N. japonica* and *N. subintegerrima*. In *Nuphar*, natural hybridization and introgression are known to cause difficulty in the taxonomic delimitation of species (Heslop-Harrison 1953; Beal 1956; Les & Philbrick 1993; Padgett *et al.* 1998, 2002). It is probable that natural hybridization also occurs widely in Japan.

Geographic distribution patterns of Clusters B and D (Fig. 3) agree well with the range of distribution of the indeterminable specimens of *Nuphar* that were provisionally identified as *N. subintegerrima* (Kadono 1994). The populations of Cluster B were within the range of overlap of *N. japonica* (Cluster A) and *N. oguraensis* (Cluster C) in western Japan. In the Hokuriku Region, however, *N. oguraensis* is not present. In contrast, populations of Cluster D are distributed more widely than one of the putative parents, *N. subintegerrima s. s.* (Cluster E). Further molecular and historical analysis of the distribution pattern of each taxon is needed to determine the origin and proper taxonomic treatment of the intermediate groups.

Nuphar oguraensis and *N. subintegerrima s. l.* have been designated as endangered species in Japan (Environmental Agency of Japan 2000). Our study shows that *N. subintegerrima s. s.* is particularly endangered. We located only three populations, all limited to the Tokai Region, although the former range was widespread in central Japan (Makino 1910). Most of the populations are extinct today (Study Group of Flora of Aichi Prefecture 1996). It has proven to be one the species most urgently in need of protection in the conservation of Japanese flora.

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